vesicles. This suggests that PC activation is more complicated than simply binding the protein to membranes. Comparing enzyme kinetics and the effects of mutations on B*t*PI-PLC, S*a*PI-PLC and the PI-PLC from an intracellular pathogen (*Listeria monocytogenes*) suggests a rationale for the acquisition of a discrete PC binding site in some, but not all, PI-PLC enzymes.

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Role of Surface Modifications in Ovalbumin Interacting with Lipid Membranes

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Recent electrophysiology studies in our laboratory suggest that some proteins from the Serine Protease Inhibitor (SERPIN) family cross cell membranes without regulated uptake mechanisms. Since these proteins do not structurally resemble typical membrane proteins, questions arise about the factors governing interactions between lipid and protein of this family. Previous studies of ovalbumin_a chicken protein of the SERPIN family_indicate that both glycosylation and phosphorylation play vital regulatory roles. Combining UV-visible and phosphorus NMR spectroscopy offers information on these posttranslational modifications. Proteins crossing the cell membrane must enter the central region of lipid tails. This hydrophobic environment causes the proteins to change conformation and altering the absorbance and resonance spectra. In UV-spectroscopy, environmental hydrophobicity cause phenylalanine, tyrosine, and tryptophan peaks to shift. In P31 NMR, the hydrophobic interactions lead to changes in phosphorus chemical shifts. Combining information from both these methods reveals the interactions of phosphorylated glycoproteins and lipid bilayers.

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Measuring Initial Insertion Forces of Ion Channels: The Impact of the Alpha-Hemolysin Phosphocholine Binding Pocket

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Alpha hemolysin (αHL) from *Staphyloccocus aureus,* spontaneously forms channels in lipid bilayers through a process of monomer aggregation and bilayer translocation. However, little is known about the forces required to initially part the outer lipid leaflet. Crystallographic studies have revealed the location of a phosphocholine binding pocket at the base of αHL's extracellular cap. As a selective gatekeeper of lipid/protein interaction, this pocket plays a putative role in the pore formation process.

Here, we explore lateral surface pressure changes in the initial stages of channel formation using a Langmuir monolayer trough. Lipid monolayers limit the process to its initial stages by prohibiting full bilayer translocation. Surface pressure results are compared to whole cell lysis experiments, where red blood cells (RBC) are used to measure the protein's ability to form fully functioning pores.

Two αHL mutations, R200A and W179R, were expressed and purified along with wild type (WT) to compare channel behavior in RBCs and in the presence of lipids containing phosphocholine (PC) or glycerol phosphate (GP). These mutations were strategically chosen to systematically alter the structure and charge interactions with headgroups in the binding pocket.

We find that W179R dramatically reduces RBC lysis. Monolayer measurements with either PC or GP show elevated or suppressed lateral surface pressures relative to WT, depending on the headgroup. In addition, the WT shows enhanced lateral surface pressures in GP lipid. These data indicate that the WT binding pocket plays a lipid-dependent role in membrane interaction. They also suggest that an anticorrelation exists between lytic ability and the monolayer area expansion. In this view, the WT creates a stable pocket around a phosphocholine head group, allowing pore formation to proceed efficiently, without expending energy unnecessarily on the separation of lipids.

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Interaction of an Acidic Peripheral Protein with Anionic Lipid Membranes: Insights from Molecular Dynamics

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The fatty acid-binding protein RePl is a soluble protein that shows the common beta-barrel motif with two alpha-helix in the portal region. Its isoelectric point of 5.85 leads to net charge of −1 at neutral pH. Previous evidence of its interaction with anionic lipids [1], makes it an attractive model to test the hypothesis that the binding and orientation of soluble proteins within lipid membranes are driven by the interaction of its macrodipole with the interphasial electric field [2].

The interaction of RePl with lipid membranes of anionic and zwitterionic phospholipids was studied by multiple-run molecular dynamics, Potential of Mean Force calculations and filtration assays. RePl has a macrodipole of 310 Debyes pointing towards the portal region. We found that it interacted selectively with anionic interphases, aligning its macrodipole in the configuration of lowest energy within the membrane electric field. Additional evidence of this orientation was achieved experimentally by FRET measurements. This orientation led to the portal region in contact with the membrane. The strength and range of the interaction and the preference in spacial configuration was attenuated by the presence of salt. A global loss of compactness was seen by MD and FT-IR spectroscopy.

A similar behaviour has been described for L-BABP [2], a protein with identical tertiary structure.

These electrostatic-like features of the interaction suggest that the interphasial electric field could be the driving force for the binding and orientation, and may be involved on the ligand delivery mechanism in this family of proteins.

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Effect of Hydrophobic Peptide Sequence upon Peptide-Dependent Acceleration of Lipid Flip-Flop

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The asymmetric distribution of different lipid species in the inner and outer half of the bilayer is an important feature of many natural membranes. Certain transmembrane (TM) helix-forming peptides are known to increase the transverse diffusion (flip-flop) of lipids and can break down lipid asymmetry. We have investigated the dependence of peptide enhanced flip-flop upon TM helix sequence in order to try to understand the mechanism by which this occurs.

The movement of a fluorescent phosphatidylcholine analog (6-NBD-PC) from the outer to inner leaflet was used to measure the influence of different model TM helices on the natural flip-flop rate of model membranes. The most hydrophobic TM peptides had very little or no effect upon flip-flop rate. Preliminary results indicate that for TM peptides of comparable length, a reduction of hydrophobicity increases the lipid flip-flop rate. The lipid flip-flop rate was most markedly increased when an Asp residue was introduced near the center of a highly hydrophobic sequence. The flip-flop was greatly accelerated when the Asp residue was protonated and the peptide was in a TM configuration but accelerated the most when the Asp residue was deprotonated and the peptide resided on the bilayer surface. Although certain pore forming peptides have been shown to accelerate flip-flop, no pore formation was detected for the peptides used in our studies. We conclude that perturbation of lipid packing is a possible mechanism by which hydrophobic helices accelerate flip-flop, while pore formation is unlikely to be a universal mechanism for accelerating flip-flop.

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Amyloid-Beta 1-42 Binds Preferentially to Nanoscale Electrostatic Domains in Cholesterol-Enriched DOPC Lipid Membrane

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The plasma membrane is a complex structure, composed primarily of phospholipids and other macromolecules, such as proteins, sterols and steroids. It plays a vital role in cell motility and acts as a barrier for extracellular materials. Amyloid fibrils are linked to multiple neurodegenerative diseases, such as Alzheimer's. Although fibril plaque formation is associated with biological membranes *in vivo*, the role of the lipid membrane in fibril formation and toxicity is not well understood. We investigated the interaction of model lipid membranes with Aβ 1-42 peptide. Using Atomic force microscopy, we demonstrated that binding of Aβ 1-42 peptide to DOPC bilayers with 20% cholesterol is non-uniform and resulted in the formation of nanoscale islands loaded with A-beta. We attribute this effect to the presence of electrostatic nanoscale domains induced by cholesterol in the DOPC bilayer. These domains were resolved by AFM imaging of the lipid bilayer and by frequency modulated Kelvin probe force microscopy in the monolayer samples prepared with the Langmuir-Blodgett monolayer technique.